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ESTABLISHMENT OF IN-HOUSE ELISA FOR SRLV DIAGNOSTICS

Maksym Samoilenko [1, 4], Elena De Martin [1, 2], Caroline Lehmann [1, 2], Antoinette Golomingi[1,2], Carlos Abril[1,2], Agata Moroz[3], Marcin Mickiewicz[3], Michal Czopowicz[3],Vitalii Nedosekov[4], Jaroslaw Kaba[3],and Giuseppe Bertoni[1,2]

[1]Institute of Virology and Immunology, Bern & Mittelhäusern, Switzerland,

[2]Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland;

[3]Division of Veterinary Epidemiology and Economics, Institute of Veterinary Medicine, Warsaw University of Life Sciences SGGW, Warsaw, Poland;

[4]The National University of Life and Environmental Sciences of Ukraine (NULES), Kyiv, Ukraine.

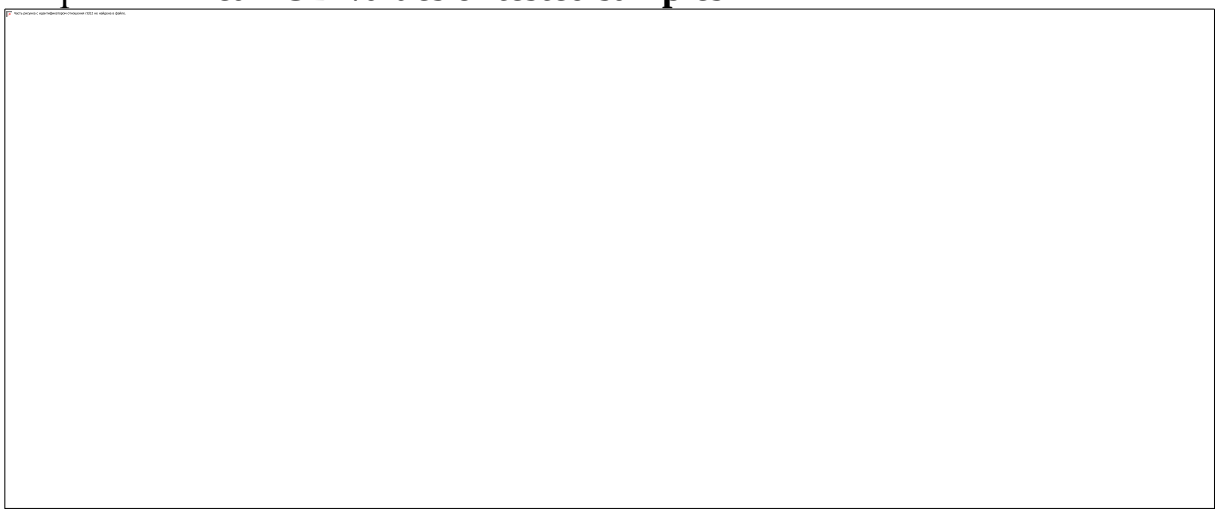
Small ruminant lentiviruses (SRLV) are a group of genetically similar viruses that cause severe chronic multisystem lesions in sheep and goats. Infections with these viruses lead to significant economic losses. SRLV group consists of 4 main subtypes: A, B, C, D, and E. The import of infected animals into Iceland led to the discovery of these viruses. The local shepherds called the disease induced by these viruses Maedi Visna (in Islandic language, Maedi stands for shortness of breath, and Visna stands for wasting). This was also the original name of the virus isolated from these sheep. Typical pathological lesions included interstitial pneumonia and inflammatory disease of the central nervous system. The virus was first isolated in 1957 on Island and became a prototypical strain of the A subtype. On the other part of the planet, another significant disease emerged in the goat population in the USA. The condition was called Caprine Arthritis Encephalitis and was characterized by the development of severe arthritis in carpal joints of infected adult goats and encephalitis in goat kids. The causative agent, named CAEV, was isolated in 1974 in the USA and became prototypical for the B subtype of SRLV. First, these two viruses were thought to be distant species, infecting sheep and goats, respectively. However, sequence analysis of numerous isolates obtained from sheep and goats indicated that Maedi Visna and CAEV strains were transmitted between the two species, classifying them as SRLV. In terms of genome organization – SRLV are pretty similar to their distant cousin – HIV. Their genome contains three main genes: *gag, pol,* and *env*. The genome also carries accessory genes such as *vpr, vif,* and *rev*. From the diagnostic point of view, the most valuable region is the *gag* because it contains several B-cell epitopes. Most commercially available diagnostic systems are based on antigens obtained from purified viral particles or are assembled by combining recombinant Gag proteins and synthetic peptides derived from the immunodominant TM3 region of the Env protein.

As proof of principle, we chose an approach based on recombinant antigens from an SRLV B prototypic strain (CAE-CO GeneBank accession number M33677). The gag region was amplified by PCR and cloned in a eukaryotic expression vector adding a secretory sequence at the N-terminus and a streptavidin tag at the C-terminus. The plasmid was amplified in *E.coli* cells, and protein expression was induced in transfected HEK cells. The obtained and purified antigen was coated on PolySorp at a 5 ug/ml concentration. Plates were blocked with 5 % dried defatted milk in PBS + 0,1% Tween 20. The following

samples were analyzed: sample 1 – goat infected with SRLV subtype A as well as subtype B; sample 2 – goat infected with SRLV subtype B; sample 3 – goat infected with SRLV subtype A; sample 4 – goat infected with SRLV subtype A; sample 5 – goat infected with SRLV subtype A. As a negative control – a reference negative sera was used. The most reliable dilution of sera proved to be 1:25.

Results Cut off point for this in-house ELISA was established by calculating the mean (x) and standard deviation of negative serum. X= 0.311, SD= 0.03. Cut off value was taken x+3SD and established at 0.314 OD. Mean values for the tested sera were: sample 1 = 1.6048, sample 2 = 1.5561, sample 3 = 0.6272; sample 4 = 0.43865; sample 5 = 0.5321. Graphical interpretation of the results is presented in the Graph №1.

Graph №1. **Mean OD values of tested samples**



These results confirmed that samples from animals infected with subtype B SRLV infection showed the highest OD values. In contrast, animals infected with A subtypes showed a weak reactivity in this system based exclusively on a B subtype, Gag protein. To further develop an SRLV diagnostic kit, a combination of SRLV subtype A and B antigens will be used.